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DEPARTMENT OF THE ARMY
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EFFECTS OF MOIST AND DRY CONDITIONING ON VIABILITY AND HATCH OF AEDES TOGOI EGGS

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ABSTRACT

Laboratory experiments were conducted to determine the effects of moist and dry conditioning on the viability and hatch of eggs of the mosquito Aedes togoi Theobald.

Maximal hatch was associated with joint contribution of moist and dry conditioning. Optimal combinations for hatch were 4 days moist and 10 to 14 days dry, 5 days moist and 10 to 14 days dry, and 6 days moist and 15 to 19 days dry.

Viability decreased as the time of moist conditioning increased, but no significant differences in viability were attributed to dry conditioning.

I. INTRODUCTION

The mosquito Aedes togoi Theobald has been colonized successfully in the laboratory, but little has been reported on the effects of moist and dry conditioning on egg viability and hatch. To avoid continuous colonization, it is desirable to store A. togoi eggs for various periods of time.

Moist conditioning is defined here as the continuous contact of the eggs with moisture under environmental conditions of 26.7±0.5 C and 80±1% RH. Dry conditioning is the continuous exposure of moist-conditioned eggs to environmental conditions of 26.7±0.5 C and 80±1% RH.

Weathersby[1] stated that the development of a single generation of A. togoi required an average of 25 days, the time from blood meal to oviposition being 5 to 6 days and the egg stage lasting 4 to 5 days. Chagin[2] concluded that the optimal temperature for development of the aquatic stages of A. togoi was within 24 to 27 C. Lien[3] maintained A. togoi eggs under saturated conditions of moisture for 4, 5, and 6 days and obtained 24-hour hatch rates of 96.7, 92.3, and 95.2%, respectively. He further demonstrated that eggs kept moist for 6 days and stored dry for 15 days hatched satisfactorily, but no percentages were recorded. Hatch was approximately 8.2% after 6 days of moist conditioning and 21 days of dry storage and unsatisfactory among eggs that had been dried for 1 month or more.

In our preliminary experiments, no statistically significant information was derived from the exposure of eggs to short periods of moist and dry conditioning. Combinations of 2 to 4 days of moist conditioning and 1 to 10 days of dry conditioning were tested, and results suggested that viability and hatch were influenced by the number of days subjected to moist conditioning. Evidence further indicated that dry conditioning for 1 to 10 days has little effect on viability, but the combination of moist and dry conditioning influences hatch. This study was designed to determine the effects of 4 to 6 days of moist conditioning and 10 to 14 days (first dry period) and 15 to 19 days (second dry period) of dry conditioning on the viability and hatch of A. togoi eggs. In this study the term viable is applied to those eggs that possess embryos.

II. MATERIALS AND METHODS

First instar larvae were obtained by immersing for 5 hours a piece of brown paper toweling containing A. togoi eggs in 1.5 cm of distilled deoxygenated water held in a white enameled pan (25.4 x 40.6 x 6.3 cm). The temperature of the hatch water was maintained at 26.7 C (80 F), and a pinch of powdered brewer's yeast was added as a hatching stimulus.

At the end of 5 hours, the larvae were counted by an aliquot method to approximate the number of larvae for each rearing tray. Approximately 10,000 larvae were introduced into each of two stainless steel rearing trays (119.72 x 63.5 x 5.08 cm) filled to a depth of 1.5 cm with 26.7 C tap water. This provided a rearing density of approximately 0.9 larvae per ml of rearing medium. The larvae were fed Jayron* raw porcine liver powder daily.

On developmental days 9 thru 13, the larvae and male and female pupae were drained from the rearing trays and separated by the glass plate method.[4] After separation, the larvae and liquid medium were replaced in the rearing trays for further pupation.

Pupae were volumetrically counted into white enameled pans (9.52 x 5.72 x 15.24 cm) at a ratio of about 6,500 males to 3,500 females. One pan of pupae was placed in each of two colony cages (53.75 x 44.5 x 44.0 cm) for adult emergence.

Emergence took place for 48 hours, after which each colony contained about 10,000 mosquitoes at a density of 1.15 adults per cm^2 of vertical resting surface.

Adults were fed a 0.3 M sucrose solution daily on cotton pads (10.16 x 30.48 cm). Four hours prior to offering a blood meal, the sucrose pads were removed, and the colonies were placed under subdued light. An anesthetized guinea pig**was then placed on top of each cage for about 3 hours and adult female mosquitoes were permitted to engorge with blood.

Three 1-pint ice cream cartons, each lined with a strip of brown paper toweling (7.62 x 27.94 cm) and filled with 26.7 C tap water to a depth of 2.54 cm, were placed inside each colony cage approximately 48 hours after the completion of engorgement. Prior to being placed in the carton, an egg paper was marked off vertically into 10 sections (2.54 x 7.62 cm) and each section was marked with the replicated number of hours subjected to moist and dry conditioning.

The oviposition cartons were left in a colony cage for 48 hours. The greatest oviposition occurred during the last 24 hours. After oviposition, the egg papers were rinsed with fresh tap water (26.7 C) to remove undesirable debris and treated with a 1:20,000 dilution of 17% tincture of Zephiran® solution to aid in the control of fungal growth during conditioning. The egg papers were cut in half vertically, and each half was placed in a separate plastic conditioning box (8.5 x 4.5 x 17.5 cm) with the clear side of the egg paper directly against moist cotton pads and the egg-laden side facing upward. The papers were held in an environmental chamber at 26.7±0.5 C and 80±1% RH.

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* Viobin Corporation, Monticello, Illinois.

** In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

The two halves of the first egg paper were removed from moist conditioning at the end of 4 days and dried for 30 minutes, and then the five previously marked sections were cut from each half. Each section contained approximately 500 eggs, estimated under a Bausch and Lomb dissecting microscope using 10X eyepieces and 6X objective. The sections of egg paper were pinned randomly to a piece of Styrofoam (98.0 x 48.0 x 2.54 cm). The Styrofoam tray of egg papers was placed for drying in an environmental chamber under controlled conditions of 26.7±0.5 C, 80±1% RH and predetermined foot-candle intensities of Gro-Lux® illumination.* Sections of egg papers were subjected to a daily cycle of 14.5 hours of illumination and 9.5 hours of darkness.

Following each 24-hour interval from 10 to 14 and 15 to 19 days, sections of egg paper were individually removed and immersed in 1-ounce vials of distilled deoxygenated water for 1 hour. Each section of egg paper was then removed from the hatching medium and returned to its respective position on the Styrofoam tray. The hatched larvae from each section of egg paper were hand-counted.

The two halves of the other two egg papers were removed from moist conditioning at the end of 5 and 6 days, respectively, and handled in the same manner.

After the initial hatch, Clorox® (sodium hypochlorite, 5.25% by weight) was applied to bleach unhatched eggs remaining on each section of egg paper. Nonviable eggs and egg shells became transparent; viable eggs showed a clear view of developed embryos.

III. RESULTS

The statistical analyses were based on three separate responses: (i) per cent total hatch (per cent hatch of total eggs); (ii) per cent total viability (per cent viability of total eggs); and (iii) per cent hatch, given viability (per cent hatch of total viable eggs).

A statistically significant joint contribution of the moist and dry conditioning periods is demonstrated in Table 1. The combinations of 4 days moist and 10 to 14 days dry, 5 days moist and 10 to 14 days dry, and 6 days moist and 15 to 19 days dry resulted in maximal total hatch. The lowest per cent total hatch was obtained from eggs subjected to moist conditioning for 4 days and dry conditioning for 15 to 19 days. The statistical analyses detected no significant differences in percentage of total hatch among the moist conditioning periods of 4, 5, and 6 days (Fig. 1), and dry conditioning periods of 10 to 14 and 15 to 19 days (Fig. 2).

* A foot-candle reading was taken at each site where an egg paper was pinned to the Styrofoam tray. The average intensity of illumination was 73 foot-candles.

TABLE 1. VIABILITY AND HATCH OF AEDES TOGOI EGGS FOLLOWING COMBINATIONS OF MOIST AND DRY CONDITIONING

Days Moist	Dry[a] Period	Total Hatch, %	Total Viability, %	Hatch Given Viability, %
4	1	67.65	94.93	70.92
5	1	67.32	91.93	73.09
6	1	58.86	87.07	67.01
4	2	48.59	93.48	51.79
5	2	58.10	93.00	63.07
6	2	64.79	87.41	73.68

a. Period 1 - Dry conditioned 10 to 14 days and hatched at 24-hour intervals.

Period 2 - Dry conditioned 15 to 19 days and hatched at 24-hour intervals.

Significant differences in per cent total viability were evident among moist conditioning periods of 4, 5, and 6 days. Moist conditioning periods of 4, 5, and 6 days resulted in 94.21, 92.50, and 87.07% viability, respectively. Therefore, as the exposure to additional moisture increased the per cent total viability decreased. No significant differences in per cent total viability occurred between the dry periods.

Statistical analyses indicated no significant differences in the percentage of eggs hatched given viability after 4, 5, and 6 days of moist conditioning, 10 to 14 and 15 to 19 days of dry conditioning. However, a significant joint effect of the two factors, moist and dry conditioning, was previously established and the combinations of 4 days moist and 10 to 14 days dry, 5 days moist and 10 to 14 days dry, and 6 days moist and 15 to 19 days dry resulted in maximal hatch given viability.

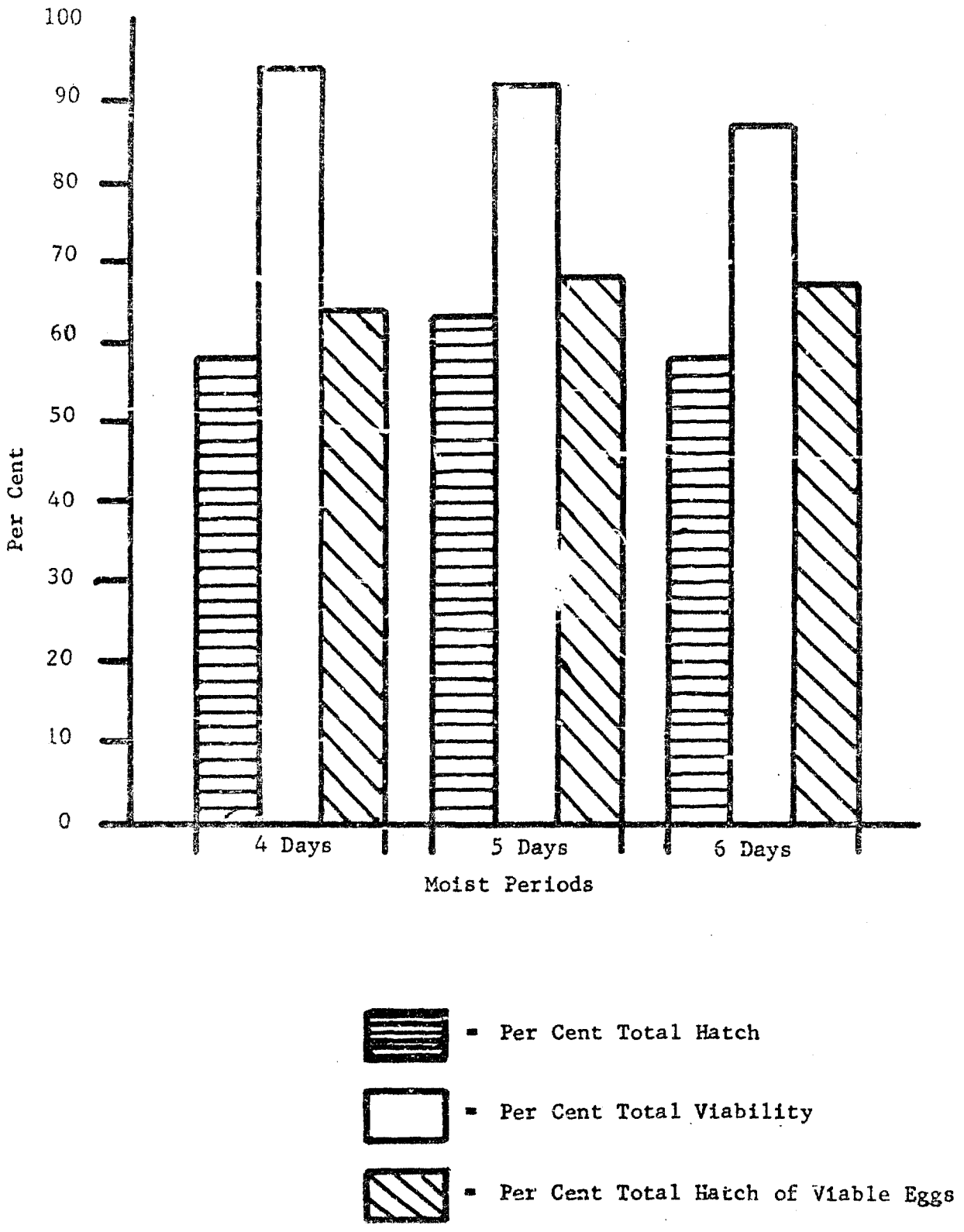

Figure 1. Viability and Hatch of Aedes togoi Eggs Following Moist Conditioning.

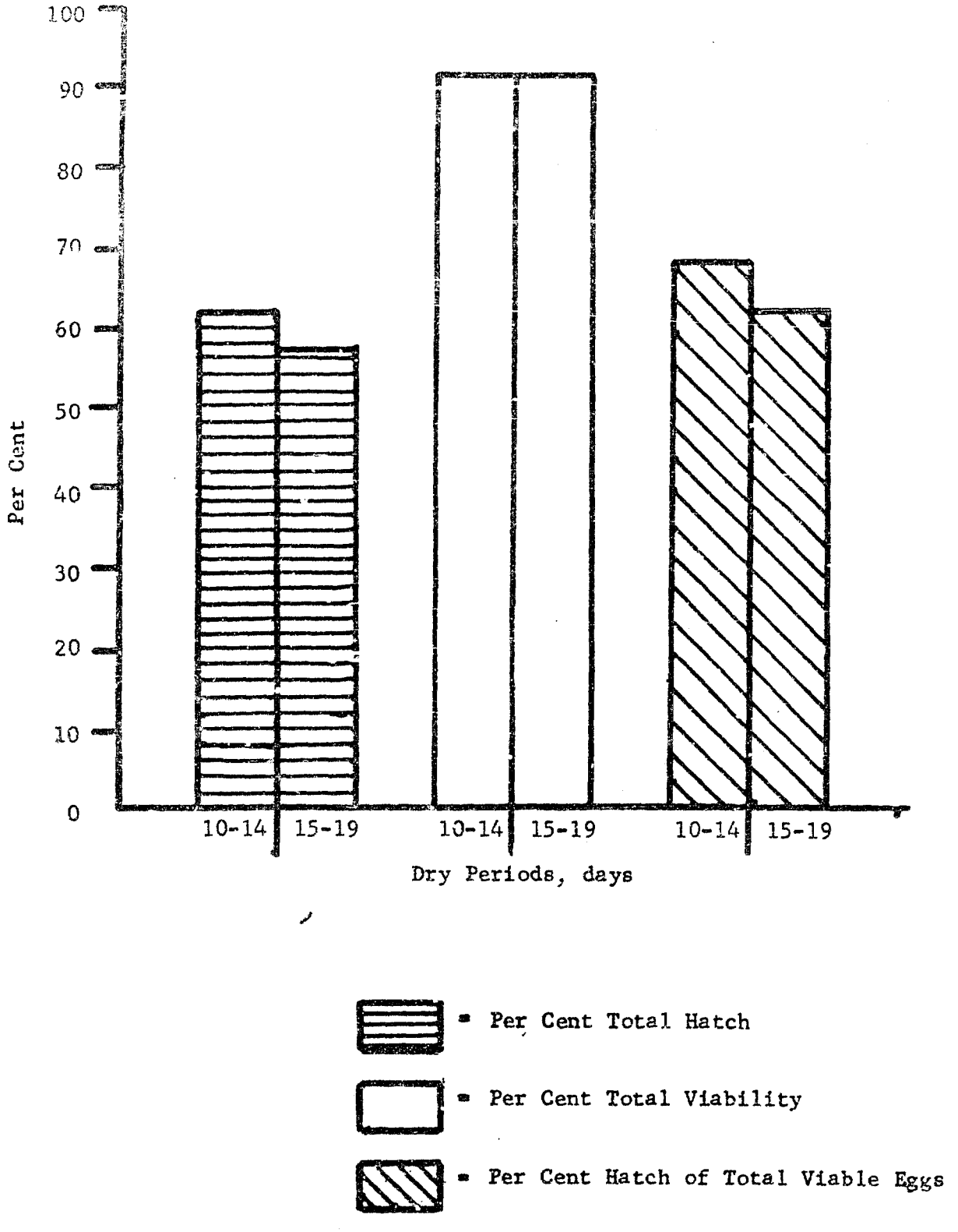

Figure 2. Viability and Hatch of Aedes togoi Eggs Following Dry Conditioning.

IV. DISCUSSION AND CONCLUSIONS

It is evident that viability and hatch are influenced by the amount of moist and dry conditioning to which A. togoi eggs are subjected. This conclusion is also suggested by the work of Lein.[3]

Although the per cent total hatch is not significantly altered by moist conditioning, it is increased as the result of a proper combination of moist and dry conditioning.

The per cent total viability is significantly affected by moist conditioning, and, as the time of moist conditioning is increased, the per cent total viability decreases. Therefore, to a large extent, viability is determined by the duration of moist conditioning. Dry conditioning has no apparent effect on total viability, either alone or in combination with moist conditioning.

A combined contribution of moist and dry conditioning increased the per cent hatch of the total viable eggs. This is also the result of a joint contribution of the two factors which closely parallels the effects of moist and dry conditioning on the per cent total hatch.

LITERATURE CITED

1. Weathersby, A.B. 1962. Colonization of six species of mosquitoes in Japan. Mosquito News 22:31-34.

2. Chagin, K.P. 1943. Observations on the development cycle of Aedes (F.) togoi under laboratory and natural conditions. Med. Parasitol. Parasitic Dis. 12:44-52.

3. Lien, J.C. 1960. Laboratory culture of Aedes (Finlaya) togoi (Theobald), 1907 and measurements of its susceptibility to insecticides. Entomol. Exp. Appl. 3:267-282.

4. Fay, R.W.; Morlan, H.B. 1959. A mechanical device for separating developmental stages, sexes and species of mosquitoes. Mosquito News 19:144-147.

REFERENCES

Edeson, J.F.B. 1962. The epidemiology and treatment of infection due to Brugia malayi. Bull. WHO 27:529-541.

Grascenkov, N.I. 1964. Japanese encephalitis in the U.S.S.R. Bull. WHO 30:161-172.

Laurence, B.R. 1964. Autogeny in Aedes (Finlaya) togoi Theobald (Diptera, Culicidae). J. Insect Physiol. 10:319-331.

Miles, J.A.R. 1964. Some ecological aspects of the problems of arthropod-borne animal viruses in the Western Pacific and South East Asia regions. Bull. WHO 30:197-210.

Nye, E.R.; Lien, J.C. 1960. Laboratory transmission of Semliki Forest virus by Aedes togoi Theo. Trans. Roy. Soc. Trop. Med. Hyg. 54:263-264.

Omori, N.; Fujii, S. 1953. On the feeding habits of Aedes togoi and some other species of mosquitoes. Yokohama Med. Bull. 4:23-31.

Petrishcheva, P.A. 1948. On the transition of Aedes togoi Theob. and Aedes japonicus Theob. (Diptera, Culicidae) to the synanthropic form of life. Intomol. Obozreniye 30:103-108.

Ramachandran, C.P.; Wharton, R.H.; Dunn, F.L.; Kershan, W.E. 1963. Aedes (Finlaya) togoi Theobald, a useful laboratory vector in studies of filariasis. Ann. Trop. Med. Parasitol. 57:443-445.

Shestakov, V.I. 1961. On the biology of the mosquito Aedes togoi Theob. Zool. Zh. 40:284-285.

Shoji, S. 1955. On the synecological observation of micro-organism communities in some tidewater rock-pools with special reference to the morphological variation of the larva of a mosquito, Aedes togoi Theobald. Ecol. Rev. 14:91-98.

Vinogradova, Ye.B. 1965. An experimental study of the factors regulating induction of imaginal diapause in the mosquito Aedes togoi Theob. (Diptera, Culicidae). Entomol. Rev. 44:309-315.